pNK2887

77343™

Description

This construct is useful for transposon mutagenesis in *Escherichia coli*. It also permits generation of promoter fusions of target genes to Plac-UV5, if the transposon inserts in the correct orientation. It contains the ats1 ats2 transposase gene that permits relaxed insertion specificity (altered target specificity, ATS). Expression is regulated by the Ptac promoter and is inducible by IPTG. The transposase segment extends from IS10R to the EcoRI site at nt 3140 of Tn10, with a deletion of nucleotides 1329-1942 to remove the transposase binding site. The construct also contains a 1.9 kb mini-Tn10 cassette conferring kanamycin resistance, bounded by inverted repeats of the outermost 70 bp of IS10R and embedded in 40 bp of lambdacI terminating in HindIII sites. It differs from pNK2859 (ATCC 77338) by also carrying a Plac-UV5 promoter in a BamHI fragment downstream of kanR and oriented so that transcription is in the same direction as the kan promoter out across the transposon end. This cassette is inserted into the HindIII site (original nt 2272) of the ATS transposase-containing EcoRI fragment of pNK2881 (ATCC 77351). Transcription from the Ptac promoter is in a direction opposite to that of amp. pBR322 was modified by deletion of bp 75-2352 and destruction (by filling in) of the HindIIII site. The order of the major features in this plasmid is: EcoRI - Ptac - ATS transposase - mini-Tn10 kan Plac-UV5 - EcoRI - pMB1 ori - ampR.Restriction digests of the clone gave the following bands (in kb): EcoRI - 4.6, 2.0; HindIII - 4.6, 1.0, 0.9; PstI - 6.4.

Organism: *Escherichia coli* (Migula) Castellani and Chalmers

Clone type: Clone

Shipping information: *Escherichia coli* HB101 containing the plasmid

Storage Conditions

Product format: Freeze-dried

Storage conditions: 2°C to 8°C

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

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Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Insert Information

Insert size (kb): 4.5
Type of DNA: genomic
Target gene: transposase; Tn10; ats1 ats2 (ATS)
Gene product: transposase, Tn10, ats1 ats2 (ATS)
Insert end: EcoRI

Vector Information

Construct size (kb): 6.599999904632568

Growth Conditions

Medium:
ATCC Medium 1227: LB Medium (ATCC medium 1065) with 50 mcg/ml ampicillin
Temperature: 37°C

Handling Procedures

Asceptically add 0.3 to 0.4 mL of liquid medium to the freeze-dried pellet and mix well. Transfer 100 *u*L to a test tube containing 5 mL LB+50mg/mL of ampicillin. A loopful of culture can also be streaked on an LB + amp agar plate. Incubate cultures at 37°C. Isolate DNA using standard plasmid preparation procedures.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: pNK2887 (ATCC 77343)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

This information on this document was last updated on 2025-09-09

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